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14. ABSTRACT

Military personnel have an increased risk of injuries that would be susceptible to infection by Staphylococci (MRSA). The approach uses the rapid killing action of a phage lysin that kills MRSA and all other staphylococci to treat MRSA-infected wounds in a rat model to prevent infection. It is anticipated that the lysin may be used in the field to eliminate MRSA during transport to the field hospital and after. We asked if combination therapy with lysin and vancomycin will be more effective in clearing MRSA from freshly contaminated wounds than the standard of care using vancomycin alone. Results show that wounds of rats treated with buffer alone exhibited 106 CFU/gram of tissue of MRSA while animals treated with both Vancomycin / lysin had an average of 102 CFU/gram of tissue, a reduction of ~4-logs of MRSA. Treatment with vancomycin/buffer or buffer/lysin resulted in a total of 105. Experiments in which a combination of vancomycin and lysin was used on established MRSA wound infections, i.e., 5-day abscesses, show that rats treated with buffer alone exhibited an average of 105 CFU/gram of tissue of MRSA while animals treated with vancomycin and lysin had an average of <102 CFU/gram of tissue, a reduction of >3-logs of MRSA. Treatment with vancomycin/buffer or buffer/lysin resulted in a total of ~103 CFU/gm, reductions of <3 logs.

15. SUBJECT TERMS

Phage lysin, MRSA, staphylococci, wound infections, field infections, hospital infections

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INTRODUCTION:

S. aureus is an opportunistic pathogen found on human skin and mucous membranes. It is the causative agent of a variety of skin and soft tissue infections in humans and serious infections such as pneumonia, meningitis, endocarditis, and osteomyelitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a cause of infections in persons within the general community (community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA)) [1]. Diseases caused by CA-MRSA range from cutaneous infection to life-threatening systemic illness [2;3]; however, the majority of disease manifests as suppurative skin and soft-tissue infections. CA-MRSA is of particular importance to the military, as soldiers are counted among the epidemiological groups who appear to be particularly at risk [4]. Military personnel have an increased risk of injuries (from skin abrasions to severe wounds) that would be susceptible to infection by these virulent bacteria, thus methods must be devised to treat them quickly and effectively. Furthermore, in the cases of severe trauma under battlefield conditions, wounds may become infected deep within relatively avascular areas. Additionally, in the process of debridement, wounds may become opportunistically infected or colonized deep within the wound that has been closed. Because many staphylococci are resistant to conventional antibiotics, treating such infections is becoming increasingly difficult [4].

The global appearance of methicillin- and vancomycin-resistant clinical isolates of *S. aureus* has become a serious concern. Currently, 40-60% of nosocomial infections of *S. aureus* are resistant to oxacillin and greater than 60% of the isolates are resistant to methicillin [5]. Treating infections caused by the drug-resistant *S. aureus* has become increasingly difficult and is therefore a major concern among healthcare professionals. To combat this challenge, development of new and effective antibiotics belonging to different classes are being aggressively pursued. A number of new antimicrobial agents such as linezolid, daptomycin, tigecyline, and ceftobiprole have been introduced or are under clinical development [6]. However, it has been reported that clinical isolates of MRSA have already become resistant to these new classes of antibiotics [7-9]. Consequently, there is an urgent need to develop novel therapeutic agents or antibiotic alternatives against MRSA.

Since the beginning of U.S. military operations in Iraq and Afghanistan, there have been more than 40,000 injuries to U.S. service members [10]. Early, aggressive, debridement is the primary tool used to fight contamination and soft-tissue injuries. Antibiotics are generally not used in early treatment, because antibiotic therapy is initiated when soldiers are admitted to U.S. military hospitals after culture and sensitivity are performed. A major source of concern is that the use of broad-spectrum antibiotics for empirical treating of combat wounds results in selection of more resistant pathogens. Also, the use of broader-spectrum agents to treat multidrug resistant infections of non-U.S. personnel in Iraq may create increasing resistance in this reservoir of patients for potential nosocomial transmission. A survey of infections from hospitals in Iraq treating combat troops

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showed that the most commonly isolated bacteria from infections in U.S. troops besides MRSA, were other staphylococci and streptococci [11]. The phage lysins will address many of these issues in that it is not an antibiotic, may be used early after the injury to control contaminating staphylococci and more importantly, it is effective against all species of staphylococci. Furthermore, the fact that lysins work synergistically with antibiotics will be useful.

Bacteriophages infect their host bacteria to produce more virus particles. At the end of the reproductive cycle (which may last up to an hour) they are faced with a problem, how to release the progeny phage trapped within the bacterium. They solve this problem by producing a peptidoglycan hydrolase enzyme called lysin that degrades the cell wall of the infected bacteria to release the progeny phage [12]. The lytic system consists of a holin [12] and at least one lysin capable of degrading the bacterial cell wall. Lysins can be endo-beta-N-acetyl-glucosaminidases or N-acetylmuramidases (lysozymes), which act on the sugar moiety, endopeptidases, which cleave the peptide cross bridge, or more commonly, an N-acetylmuramoyl-L-alanine amidase (or amidase), which hydrolyzes the amide bond connecting the sugar and peptide moieties. Typically, the holin is expressed in the late stages of phage infection, forming a pore in the cell membrane, allowing the preformed lysin(s) to gain access to the cell wall peptidoglycan, resulting hypotonic lysis of the cell releasing phage progeny. Significantly, exogenously added lysin can lyse the cell wall of uninfected cells, producing a phenomenon known as lysis from without. However, because of the lack of an outer membrane, this event is observed only in gram-positive bacteria.

While lysins have been known for many years [13-15], our laboratory was the first to use these enzymes therapeutically and prophylactically *in vivo* in their purified form to kill colonizing pathogenic bacteria on mucous membrane surfaces, infected tissues and in blood. So long as contact can be made with the bacteria, lysins have the capacity to kill the cell. In general, lysins are specific for the bacterial species from which they were produced, resulting in targeted killing. For example, we have purified lysins to kill *S. aureus* (MRSA), *S. pyogenes [16] S. pneumoniae [17]*, Group B streptococci [18], *Enterococcus faecalis/faecium* [19] and *B. anthracis [20]*. All of these enzymes are highly evolved molecules designed for a specific purpose, to quickly destroy the bacterial cell wall. Nanogram to sub-microgram quantities of purified lysin per milliliter is sufficient to sterilize a 10^7 bacterial suspension in seconds to minutes. To date, other than chemical agents, there is no biological compound known that can kill bacteria this quickly. Since nearly all bacteria are or can be infected by bacteriophage, such enzymes may be developed for nearly all disease-causing gram-positive bacteria.

BODY:

I. ClyS Lysins and Vancomycin in a 10-Day Wound Infection Model

We began our studies with animal experiments to determine the effects of the combination of vancomycin and lysin on MRSA wound infections. In these experiments we asked if the combination therapy will be more effective in clearing MRSA from the infected wounds than the standard of care using vancomycin alone.

Treatment:
Day 0 - Rats were surgically opened and infected with MRSA (strain MW2) as described in previous reports.

Day 4 - Rats started treatment with 50mg/kg every 12 hours IP with vancomycin. This treatment with vancomycin continued for the 10 days of the experiment.

Day 5 - (36 h after vancomycin began) rat wounds / infections were re-opened, debrided and drained by wiping with sterile gauze and scalpel scraping

Wounds were then washed with 500ul of 10mg/ml ClyS or Buffer before closing with surgical staples.

Day 10 - Animals were Euthanized, wounds reopened, swabbed, tissue samples of infected muscle and/or abscesses were collected, disrupted and plated for CFU/gram.

Results. As can be seen in Figure 1, the wounds of rats that were treated with buffer alone exhibited an average of 5.41×10^6 CFU/gram of tissue of MRSA while animals treated with Vancomycin / ClyS lysin had an average of 8.86×10^2 CFU/gram of tissue, a reduction of ~4-logs of MRSA. Treatment with vancomycin/buffer or buffer/ClyS resulted in a total of 1.37×10^5 and 9.44×10^5 respectively, intermediate reductions of about 1- 2 logs.

These experiments will be repeated with more animals to determine reproducibility and statistical power. However, these results are consistent with the idea that lysins synergistically with antibiotics to effectively kill MRSA. We plan to repeat these experiments and use an ointment formulation of the lysins in wound infections to determine if efficacy can be increased.
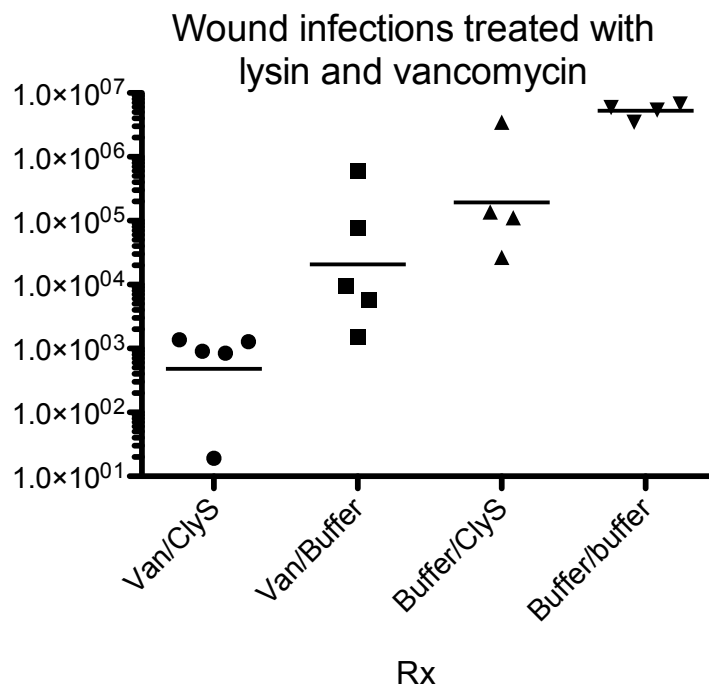
Wound infections treated with lysin and vancomycin

Figure 1. MRSA wound infections were started on day 0, then, on day 4 vancomycin treatment or buffer was initiated and continued every 12 h for 10 days. On day 5 wounds were opened and debrided and treated with 500 ul of 10 mg/ml of ClyS lysine or buffer before closing. On day 10 all animals were euthanized and tissues removed and processed to obtain colony counts.

Raw data:

	Vancomycin/ClyS	CFU/gram	Vancomycin/buffer	CFU/gram	Buffer/Clys	CFU/gram	Buffer/buffer	CFU/gram
	— IP Vancomycin + ClyS wash upon draining and debridement of wound							
	1	9.10E+02	1	5.85E+03	1	2.67E+04	1	5.42E+06
	2	1.92E+01	2	9.46E+03	2	1.12E+05	2	6.76E+06
	3	8.49E+02	3	7.81E+04	3	1.38E+05	3	3.49E+06
	4	1.37E+03	4	1.51E+03	4	3.50E+06	4	5.95E+06
	5	1.28E+03	5	5.92E+05				
Av		8.86E+02		1.37E+05		9.44E+05		5.41E+06

II. Effects of Lysin and/or Vancomycin Treatments in a 10-Day Established MRSA Wound Infection

In this this set of experiments we continued our animal experiments to determine the effects of the combination of vancomycin and lysin on *established* MRSA wound infections. This differs from our previous results, where lysin was added early after contamination with MRSA (as would occur in the field) where we clearly show >4-log reduction compared to control given the high 10^7 dose of MRSA. In the current set of experiments we use lysin alone or in combination with Vancomycin to treat an established infection (abscess formation after 5 days) and compare it to the standard of care, Vancomycin alone.

Treatment:

Day 0 - Rats were surgically opened and infected with MRSA (MRSA strain MW2) as described in previous reports.

Day 4 - Rats started treatment with 50mg/kg every 12 hours IP with vancomycin. This treatment with vancomycin continued for the 10 days of the experiment.

Day 5 - (36 h after vancomycin began) rat wound infections were re-opened, debrided and drained by wiping with sterile gauze and scalpel scraping.

Wounds were then washed with 500ul of 10mg/ml ClyS or buffer before closing with surgical staples.

Day 10 - Animals were euthanized, wounds reopened, swabbed, tissue samples of infected muscle and/or abscesses were collected, disrupted and plated for CFU/gram.

Results. As seen in Figure 2, the wounds of rats that were treated with buffer alone exhibited an average of 2×10^5 CFU/gram of tissue of MRSA while animals treated with Vancomycin and PlySS2 lysin had an average of $<10^2$ CFU/gram of tissue, a reduction of >3-logs of MRSA. Treatment with vancomycin/buffer or buffer/PlySS2 resulted in a total of ~10^3 CFU/gm, reductions of <3 logs.

It should be noted that 6/16 animals in the PlySS2/Vanco group and 3/16 in the PlySS2 alone group showed no CFUs at 10 days, so 1 CFU was used for the analysis. Importantly, the results show that one dose of PlySS2 alone on day 5 was more effective than 5 days of treatment with Vancomycin alone. This result also emphasizes the usefulness of lysin treatment (wound irrigation) during the debridement process. Furthermore, a dose of 1×10^7 MRSA was used to infect the wounds, far more than would be expected to contaminate a wound in the field.
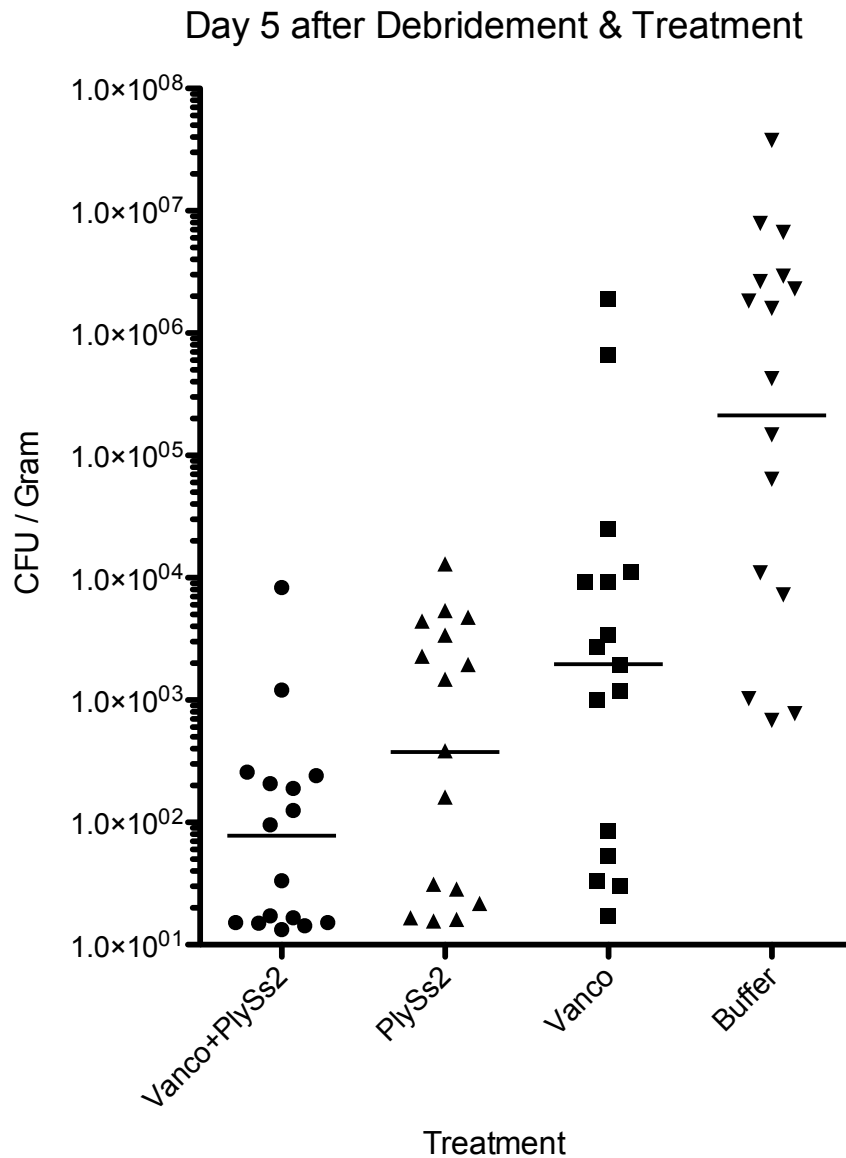
Figure 2. MRSA wound infections were started on day 0 and on day 4 vancomycin treatment or buffer was initiated and continued every 12 h for 10 days. On day 5 wounds were opened and debrided and treated with 500 ul of 10 mg/ml of PlySS2 lysin or buffer before closing. On day 10 all animals were euthanized and tissues removed and processed to obtain colony counts. Based on Mann-Whitney analysis   p=0.0001 between buffer and the combination of Vanco and PlySS2, as was PlySS2 alone and buffer.

III. Effects of Lysin on high and low dose of MRSA-infected wounds and effects of formulated lysin in a gel on clearance of MRSA-infected wounds.

In our previous experiments we used an inoculum of MRSA of around 10^7 CFU to establish abscesses in the rat wounds. Unfortunately, this may not mimic the exposure to MRSA that may be acquired on the battlefield, which is likely much lower. To address this here, we repeated our lysin treatments using MRSA doses of 10^4 CFU and compared it to wounds infected with 10^7 MRSA. Also, we have developed a topical formulation gel that we find increases stability, is easier to manipulate and increases the residence time of the lysin in the wounds. This formulated lysin was tested in our regular wound infection model.

A. Low vs High Dose of MRSA in wounds

Treatment:

Day 0 - Rats were surgically opened and infected with low (10^4) and high (10^7) dose of MRSA (strain MW2) as described in previous reports. 15 minutes later the wounds were treated with 500ul of 10mg/ml PlySS2 lysin and the wounds stapled shut.

Day 5 - Animals were euthanized, wounds reopened, swabbed, tissue samples of infected muscle and/or abscesses were collected, homogenized and plated for CFU/gram.

Results. As seen in Figure 3, the wounds of rats that were infected with low dose (10^4) of MRSA and treated with buffer alone exhibited an average of 5×10^4 CFU/gram of tissue of MRSA. The variation is large in this untreated group because this low dose can be somewhat handled by the animals. However, we could recover no CFUs in all the animals treated with PlySS2 in this low dose group. While the CFUs in the high dose MRSA group treated with buffer all clustered around 10^8 CFUs, 8/11 of the lysin-treated group were below our level of detection. Thus, it is expected that wounds contaminated in the field with MRSA would be around the 10^4 CFUs or less used in this experiment. Our results show that these wounds would easily be sterilized of these pathogens with one treatment of lysin. Even at doses up to 3-logs higher of MRSA, the single dose of lysin was able to remove all the MRSA in 72% of the infected wounds.

B. Using slow release formulated lysin in MRSA wound infections

Day 0 - Rats were surgically opened and infected with (10^7) dose of MRSA (strain MW2) as described in previous reports. The wounds were then treated with 1 ml of 5 mg/ml of PlySS2 lysin formulated in 2% methyl cellulose and 10% glycerol gel and the wounds were stapled shut.

Day 5 - Animals were euthanized, wounds reopened, swabbed, tissue samples of infected muscle and/or abscesses were collected, homogenized and plated for CFU/gram.

Result:

As can be seen in Figure 4, CFU counts clustered around 5×10^7 in the animals treated with formulated buffer, while 7/11 animals treated with formulated lysin had counts below our detectible limit. This result is comparable to the use of liquid lysin and shows that this type of formulation does not have an adverse effect on the lysin and as such we will be able to better handle and manipulate the lysin in this formulation rather than in a liquid.
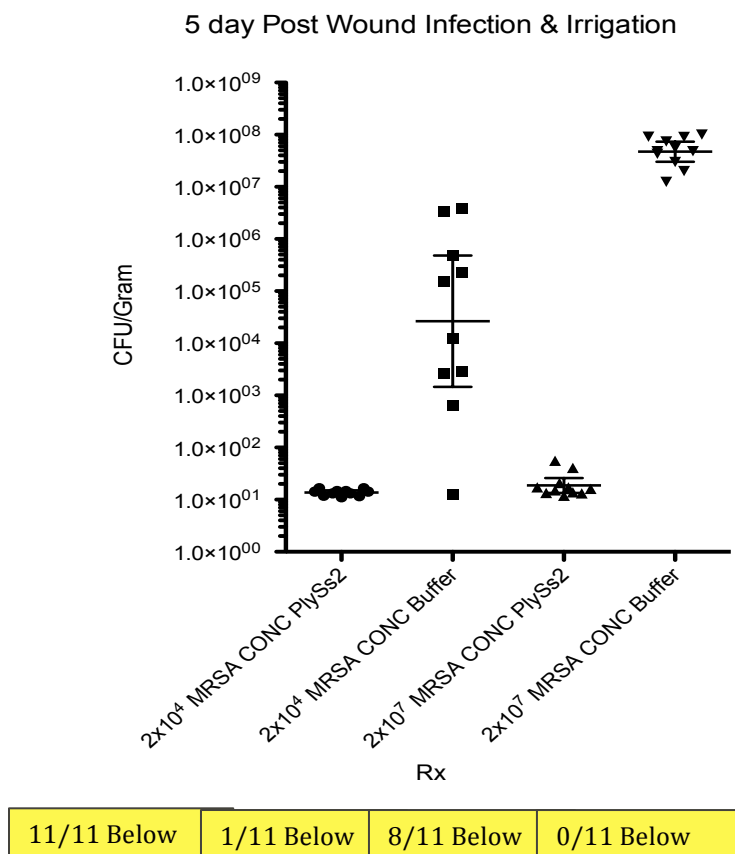

| 11/11 Below | 1/11 Below | 8/11 Below | 0/11 Below |

Figure 3. Comparison of high and low dose of MRSA after treatment with PlySS2 lysin. Numbers "Below" in yellow = animals with counts below detectible limits)
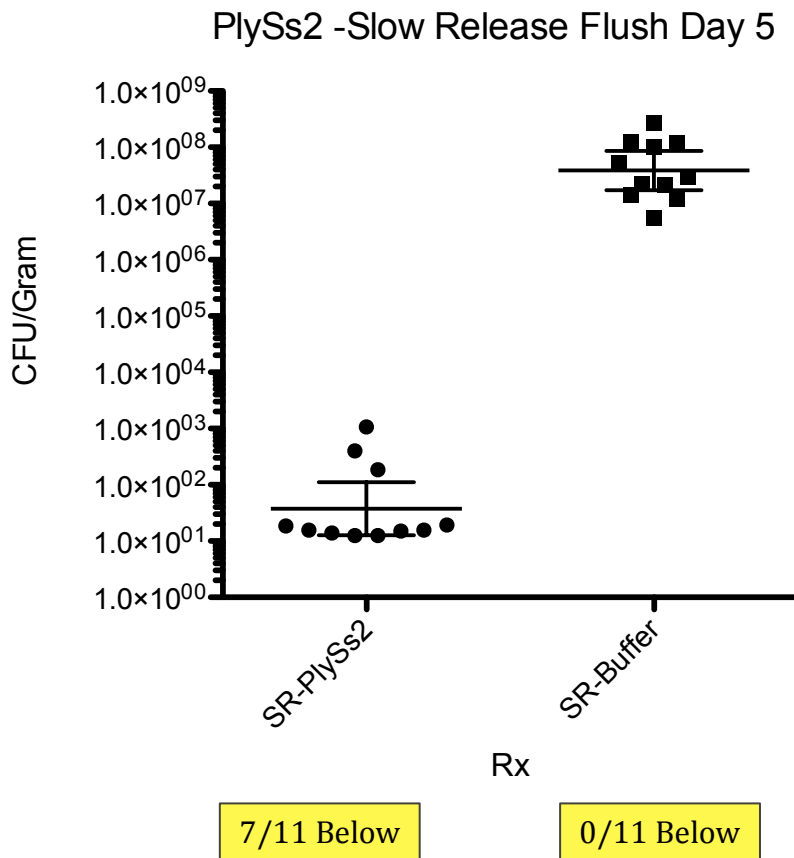
PlySs2 -Slow Release Flush Day 5

7/11 Below	0/11 Below

Figure 4. The ability of formulated lysin in a 2% methyl cellulose and 10% glycerol gel on the clearance of MRSA from infected wounds.

IV. Treatment of mixed bacterial infections with lysins

Killing both MRSA and *S. pyogenes* using a single lysin. Since the PlySS2 lysin we have been using in all of our wound studies not only kills MRSA effectively, but also has a similar effect on *S. pyogenes*, an organism that also contributes to the wound infections occurring on the battlefield and in military training camps. We examined whether a single lysin having both activities, will be able to control an infection by both pathogens. In order to show this more effectively we developed a model of lethal bacteremia in mice infected by both MRSA and *S. pyogenes*, and attempted to control both these infections with a single lysin.

First we needed to establish the dose of each pathogen that would cause disease on its own and then used the combination of both bacterial infectious doses in the final experiment. As treatment and controls we used the following lysins that are either specific for each organism or the broad acting PlySS2:
PlyC – is specific for *S. pyogenes* and will not kill MRSA
ClyS – is specific for MRSA and will not kill *S. pyogenes*

PlySS2 – has broad activity and will kill both MRSA and *S. pyogenes*

Experiment: Mice received 10^6 MRSA and / or 10^7 *S. pyogenes* intraperitoneally (IP) and after 3 hours all animals were bacteremic (based on preliminary experiments). At this time animals were treated IP with:
1. PlySS2 alone
2. ClyS + PlyC
3. ClyS alone
4. PlyC alone
5. Buffer

All animals were followed for 7 days for survival.

Results: As can be seen in Fig 5, animals with a mixed infection and treated with either PlySS2 or the combination of the PlyC and ClyS lysins were protected from death (left panel). Treatment with only one of the lysins caused death by the second organism. Animals infected with only one of the two pathogens (center and right panels) could be protected with PlySS2 lysin or the single lysin specific for the infecting organism. These results strongly indicate that not only can a lysin with broad activity be used in our wound infection model, but that a mixture of 2 lysins with different lethal specificities can be as effective in preventing infection.
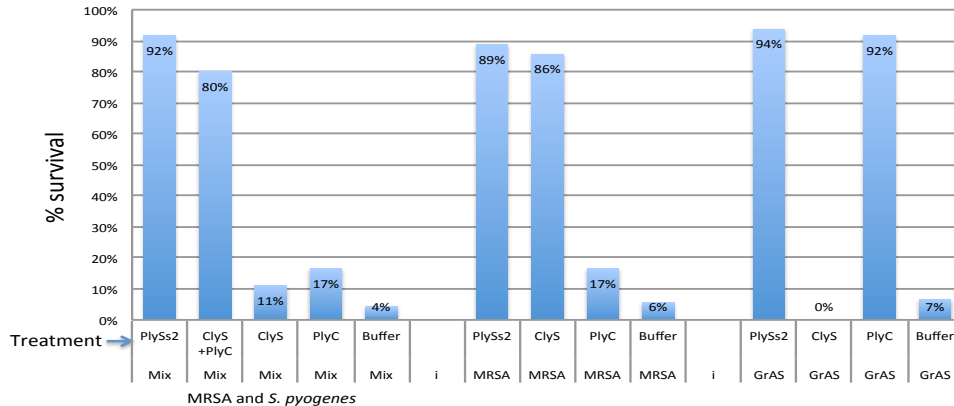

Figure 1. Protection from Death After Dual Infection with MRSA and *S. pyogenes.* Mice received 10^6 MRSA and / or 10^7 *S. pyogenes* IP, and after 3h they were treated with either PlySS2 (n=24), ClyS + PlyC (n=18), ClyS (n=20), PlyC (n=20), or buffer (n=24).

KEY RESEARCH ACCOMPLISHMENTS

1. We have established a reliable rat model of wound infection by MRSA
2. We have determined that the addition of a foreign body allows for a reduction in the number of MRSA to cause an abscess
3. We have found that lysin may be used to kill MRSA in a contaminated wound
4. We have found that the combination of vancomycin (the standard of care drug) works synergistically with lysin to achieve a better outcome in the reduction of CFUs of MRSA
5. We have found that formulating the lysin in a slow release form also works to control abscess formation

REPORTABLE OUTCOMES:

1. Manuscript in preparation
2. A reliable animal model was developed
3. The MRSA-specific lysins have been licensed by Contrafect Corporation, Yonkers, NY. The lysin is being developed to treat hospital MRSA sepsis

CONCLUSIONS. We have clearly shown that phage lysins may be used in wounds to control MRSA from causing infections and abscesses. We show that lysin works more effectively than vancomycin alone and can be used to irrigate wounds that are infected with MRSA to eliminate the pathogen. We also show that a single lysin that has activity against both MRSA and *S. pyogenes* is able to control both pathogens. In anticipation of formulation studies, we also show that a gel formulation used in the wounds works well in clearing the MRSA and is better manipulated for that application. Thus, based on our animal studies, we are confident that lysins may be used in a field application to prevent infection by MRSA and perhaps also *S. pyogenes*. It may also be used as a topical treatment of the surface of sutured wounds to prevent infections in a hospital environment.

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2012	Medical Grand Rounds, Weill Cornell Medical College, NY
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2011	Keynote address, Phage 2011, Oxford England
2011	Edwin H. Beachey Distinguished Visiting Professorship, UT, Memphis
2011	Keynote address, Dutch Infectious Diseases Update Course, Amsterdam
2010	Invited Speaker, Institute Pasteur Phage Symposium
2010	Honorary Doctor of Science, Wagner College, Staten Island, NY
2009	Invited Speaker, Nobel Symposium – Sweden "*Gram-Positive Infections"*
2009	Distinguished Nelson Lecturer, University of Montana
2008	Speaker: Royal Society London,"
2008	Keynote Speaker, Edinburgh International Phage Conference
2007	Keynote Speaker, Pennsylvania ASM
2007	Distinguished lecture, CDC, Atlanta
2007	OKU Distinguished Lecture, NYU College of Dentistry
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2004	McLaughlin Lecturer, University of Texas, Galveston
2004	ASM Lecturer, American Society of Virology, Montreal
2003	Ellison Medical Foundation Lecture (Wind River Conference, CO)
2003	State of the Art Lecture, Am. Society of Virology, Davis, CA
2003	Chair, Gordon Conference, Chemical and Biological Terrorism Defense
2003	Invited speaker, ASM Biodefense Conference, Baltimore, MD
2002	Invited speaker, Banbury meeting on Bacteriophage Biology, CSH, NY
2002	Invited speaker, Boston University, Boston, MA
2002	Cover article - Nature, Aug. 22. "Defense against Anthrax"
2002	Keynote address, International Organization for Mycoplasmology
2001	Guest speaker, Swiss Society of Intensive Medicine
2000	Keynote address, Joint German Conference for Microbiology
1999	Keynote address, Japanese Lancefield Society Annual Meeting
1999	John H. Hanks Memorial Lecture, Johns Hopkins School of Public Health
1999	Pfizer Lectureship (U. Pittsburgh)
1997 - 2007	**MERIT Award**, National Institutes of Health
1996 - 1997	Foundation Lecturer, American Academy for Microbiology
1996	Keynote address, International Lancefield Society, Paris
1995	Invited speaker, Institut Pasteur Symposia, "The Year of Louis Pasteur"
1994 - Pres	Fellow, American Academy for Microbiology
1994 - 1995	Chairman, Div. B (Microbial Pathogenesis), Am. Society for Microbiology
1992	McLaughlin Lecturer, University of Texas, Galveston
1992	Burroughs Wellcome Visiting Professor, (University of Arizona)
1987 - 1997	**MERIT Award**, National Institutes of Health
1987	Invited speaker, Institut Pasteur Centennial
1987	Shipley Lecturer, Harvard Medical School
1986 - 1987	President, Lancefield Society
1980	Alumni Achievement Award, Wagner College
1977 - 1982	Research Career Development Award, National Institutes of Health
1973 - 1977	Senior Investigator, New York Heart Association
1971 - 1973	Helen Hay Whitney Foundation Fellowship
1970	NYU Founders Day Award for outstanding scholarship

Professional Activities (selected):

2012 – Pres	Chairman, Scientific Advisory Board, Avacyn Corp
2012 - Pres	Associate Editor, Microbiology Spectrum, an ASM publication
2010 – Pres.	Chairman, Scientific Advisory Board, ContraFect Corp.
2009 – Pres.	ASM Press Books Committee
2008 – Pres.	Advisory Board: The Center for Structural Genomics of Infectious Diseases
1994 – Pres.	Board of Scientific Advisors and Trustee, Trudeau Institute
1992 – Pres.	Advisory Editor, Trends in Microbiology
1990 – Pres.	Advisory Editor, Journal of Experimental Medicine
2009 - 2010	Advisory Board: DTRA (Defense Threat Reduction Agency)
2004 - 2008	Scientific Advisory Board, Great Lakes Regional Center of Excellence
2002- 2010	Chairman, Scientific Advisory Board, Enzybiotics, LLC
1999 - 2019	Microbiology Advisory Board, New York Academy of Sciences
1986 - 1996	Advisory Board, New York Hall of Science
2000 - 2003	Awards Advisory Board, American Society for Microbiology
1996 - 2002	Scientific Advisory Board, SIGA Technologies
1999 - 2002	Chairman, Institutional Review Board (IRB), Rockefeller University
1996 - 2000	Advisory Board, Defense Advanced Research Projects Agency (DARPA)
1996 - 2001	Chief Scientific Advisor, SIGA Pharmaceuticals
1989 - 1999	**Editor-in-Chief,** Infection and Immunity
1984 - 1998	Co-Director, Biotechnology Facility at the Rockefeller University

1995 - 1997   Member, Scientific Advisory Board, Spectral Diagnostics
1993 - 1995   Chairman, Scientific Advisory Board, M6 Pharmaceuticals
1988 - 1989   Assistant Editor, Journal of Experimental Medicine
1980 - 1985   Section Editor, Journal of Immunology
1978 - 1983   NIAID Bacteriology and Mycology Study Section (BM2)
1978 - 1989   Editorial Board, Infection and Immunity
1978 - 1980   Editorial Board, Journal of Immunology

Patents: **US   Patent No.**

1. Production of streptococcal M protein immunogens — # 4,784,948
2. Streptococcal immunoglobulin A binding protein — # 5,352,588
3. Immunoglobulin binding protein ML2.2 — # 5,556,944
4. Regulation of exoproteins in *Staphylococcus aureus* — # 5,587,288
5. Method for exposing group A streptococcal antigens and an improved diagnostic test for the identification of group A streptococci — # 5,604,109
6. Polypeptide of a hybrid surface protein by bacteria — # 5,616,686
7. Process, apparatus and reagents for isolating cellular components — # 5,634,767
    (Commercialized as "Fast-prep" RNA/DNA isolation system by Q-biogene)
8. Gene for serum opacity factor — # 5,707,822
9. Delivery and expression of a hybrid surface protein by bacteria — # 5,786,205
10. Use of Gram-positive bacteria to express recombinant proteins — # 5,821,088
11. Production of streptococcal M protein — # 5,840,314
12. Fibronectin/fibrinogen binding protein of group A streptococci — # 5,910,441
13. Method for screening inhibitors of the enzyme which cleaves the anchor of surface proteins from Gram-positive bacteria — # 5,968,763
14. Regulation of exoprotein in Staphylococcus aureus II — # 5,976,792
15. Prophylactic and therapeutic treatment of group A streptococcal infections — # 5,985,271
16. Recombinant poxvirus and streptococcal M protein vaccine — # 5,985,654
17. Therapeutic treatment of group A streptococcal infections — # 5,997,862
18. Therapeutic treatment of group A streptococcal infections — # 6,017,528
19. Topical treatment of streptococcal infections — # 6,056,955
20. Use of bacterial phage associated lysing enzymes for the prophylactic and therapeutic treatment of various illnesses — # 6,056,954
21. Plasmin binding protein and therapeutic use thereof — # 6,190,659
22. Use of bacterial phage associated lysing enzymes for treating various illnesses — # 6,238,661
23. Bacterial phage associated lysing enzymes for treating dermatological infections — # 6,248,324
24. Use of phage associated lytic enzymes for treating bacterial infections of the digestive tract — # 6,254,866
25. Parenteral use of bacterial phage associated lysing enzymes for the therapeutic treatment of bacterial infections — # 6,264,945
26. Composition incorporating bacterial phage associated lysing enzymes for treating dermatological infections — # 6,277,399
27. Use of bacterial phage associated lysing enzymes for treating streptococcal infections of the upper respiratory tract — # 6,326,002
28. Receptor for Mycobacterium leprae and methods of use thereof — # 6,331,405
29. Use of bacterial phage associated lysing enzymes for treating bacterial infections of the mouth and teeth — # 6,335,012
30. Receptor for *Mycobacterium leprae* and methods of use thereof — # 6,331,405
31. Fibronectin/fibrinogen binding protein of group A streptococci — # 6,355,477
32. Composition for treatment of a bacterial infection of the digestive tract — # 6,399,097
33. Composition for treating dental caries caused by *S. mutans* — # 6,399,098
34. Composition for treatment of ocular bacterial infection — # 6,406,692
35. Composition for treatment of a bacterial infection of upper respiratory tract — # 6,423,299
36. Vaginal suppository for treating group B streptococcal infections — # 6,428,784
37. Use of bacterial phage associated lysing enzymes for treating

dermatological infections	# 6,432,444
38. Synthetic peptides from streptococcal M protein and vaccines prepared therefrom	# 6,602,907
39. C1 bacteriophage lytic system	# 6,608,187
40. Chewing gum containing phage associated lytic enzymes for treating streptococcal A infections	# 6,685,937
41. Method for the treatment of bacterial eye infections	# 6,875,431
42. Tampon for the treatment of streptococcal group B infections	# 6,881,403
43. Therapeutic treatment of upper respiratory infections using a nasal spray	# 6,893,635
44. Method of treatment of vaginal infections	# 6,899,874
45. Nasal spray for treating streptococcal infections	# 7,014,850
46. Syrup composition containing phage associated lytic enzymes	# 7,063,837
47. Use of bacterial phage associated lysing enzymes for treating upper respiratory illness	#7,141,241
48. Phage-associated lytic enzymes for the treatment of *Bacillus anthracis* and related conditions	#7,402,309
49. Phage associated lytic enzymes for treatment of pneumonia	#7,569,223
50. Nucleic acid and polypeptides of C1 bacteriophage and uses thereof	#7,582,729
51. Bacteriophage lysins for *Enterococcus faecalis*, *Enterococcus faecium* and other bacteria	#7,582,281
52. Glycosylated LPXTGases and uses thereof	#7,604,975
53. PlyGBS mutant lysin	#8,105,585

Societies: American Society for Microbiology, Kunkel Society,
New York Academy of Sciences